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Pharmacokinetics of Fenvalerate after Intravenous Administration to Sheep

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Abstract: The pharmacokinetics of total radioactivity and of intact fenvalerate were determined in sheep treated intravenously with radiolabelled or non-radiolabelled fenvalerate. Mean residence times (MRT) of total radioactivity and intact fenvalerate in plasma were 910 (±75) and 39 (±3) min, while harmonic mean elimination-phase half-lives (TM_β) were 990 and 82 min, each respectively. Systemic clearance values (Cl_S) of total radioactivity and intact fenvalerate were 2·8 (±0·3) ml min^{-1} kg^{-1} and 51·3 (±5·9) ml min^{-1} kg^{-1}, respectively. Volumes of distribution at steady state (V_{SS}) were each near 2500 ml kg^{-1}. Elimination of radioactivity occurred, in part (33·3 (±3·3)% of dose), by renal excretion, at a rate (0·9 (±0·1) ml min^{-1} kg^{-1}), similar to that of glomerular filtration. These data are consistent with a disposition model according to which intact fenvalerate was rapidly distributed into a peripheral compartment, where metabolism occurred. In addition, since the elimination half-life of fenvalerate from plasma was less than 90 min after intravenous injection, 'flip-flop' kinetics should be considered when longer elimination half-lives are observed after oral or dermal exposures.

Key words: fenvalerate, pharmacokinetics, sheep.

1 INTRODUCTION

Pyrethroid insecticides, such as fenvalerate, are thought to be rapidly metabolized and quickly eliminated from animal tissues.[1,2] However, studies typically involve oral or dermal treatments and little information is available on the pharmacokinetics of pyrethroids after intravenous administration. Non-instantaneous entry into the systemic circulation can complicate interpretation of pyrethroid pharmacokinetics since the percentage of dose absorbed is often unknown. Prolonged absorption can also result in 'flip-flop' kinetics,[3,4] a condition under which absorption and elimination rate constants can be confused. For example, after oral dosing, absorption of fenvalerate by goats appeared to be rapid, with an apparent half-life of absorption near 2 h.[5] In sheep, however, absorption of *cis*-cypermethrin was more prolonged and maximum concentrations in plasma were not attained until 24 h after treatment.[6] Determination of the pharmacokinetics of an intravenously administered pyrethroid could help explain these differences.

The purpose of the present experiments was to determine the pharmacokinetics of intravenously administered fenvalerate in sheep. Such data might allow some greater characterization of the disposition of fenvalerate in this species and, at the same time, demonstrate an elimination half-life uncomplicated by absorption processes.

2 MATERIALS AND METHODS

2.1 Materials

[*Carbonyl* ^{14}C]-radiolabelled (4·4 mCi mmol^{-1}, 163 MBq mmol^{-1}) and non-radiolabelled fenvalerate (technical grade) were obtained from Shell Research Ltd, Sittingbourne Research Centre, Sittingbourne, Kent and Ciba Geigy Canada Ltd. Radiolabelled material was further purified by preparative thin layer

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chromatography on a silica gel layer in the solvent system: toluene + hexane + glacial acetic acid (75 + 15 + 10 by volume). Nonradiolabelled fenvalerate was employed without further purification. Solvents were HPLC grade.

2.2 Animal procurement and preparation

Six Suffolk ewes (bodyweights 51 (±8) kg, range 39 to 63 kg) were obtained from the Canada/Alberta Livestock Research Trust flock, Lethbridge Alberta. Ewes were housed in outdoor facilities with access to enclosed shelter and were moved to the laboratory 24 h prior to conduct of experiments. Approximately one hour prior to treatment cannulae were inserted into the left and right jugular veins. Solutions of fenvalerate were filtered (0·45 μm) and were administered through the left cannula. Plasma was collected, through the right cannula, at the times indicated in the figures. Immediately after collection, blood was centrifuged and plasma was separated and frozen at −40° until analysis.

2.3 Dosing procedures

2.3.1 Radioactive fenvalerate

Dose solutions for experiments with radioactive fenvalerate were prepared by dissolving the required weight (1 mg kg^{-1} of animal bodyweight) of nonradiolabelled fenvalerate in polyethylene glycol-200 (0·05 ml kg^{-1} of bodyweight) containing [^{14}C]fenvalerate (0·1 mCi, 3·7 MBq). Appropriate volumes of the dose solutions were administered through the left jugular canula over a three-minute injection period. Exact doses were determined by assaying each dose solution for radioactivity and subtracting any radioactivity remaining in the container and syringe after dosing.

Blood was collected through the right jugular cannula, into vacutainers containing potassium fluoride/sodium oxalate anticoagulant, at times indicated in the figures. Urine was allowed to pass through the metabolism cage floor and was collected during the periods 0–12, 12–24, 24–48, 48–72 and 72–96 h after treatment. No attempt was made to correct for fecal contamination. Each urine volume was recorded. Plasma and aliquot portions of urine were retained at −40°C until analysis.

2.3.2 Non-radioactive fenvalerate

Filter-sterilized (0·45 μm) solutions of non-labelled fenvalerate were prepared in ethanol + polyethylene glycol-200 (2 + 1 by volume, 15 ml) and were infused via the left jugular, at a rate of 1 ml min^{-1} over a 15-min infusion period (0·133 mg kg^{-1} min^{-1}, 2 mg kg^{-1}) employing a syringe infusion pump (Harvard Apparatus, Millis, Mass). Blood was collected through the right jugular at the times indicated in the figures. All collections were timed relative to the start of the infusion.

2.4 Analytical techniques

2.4.1 Total radioactivity

Total radioactivity in plasma (3 × 0·5 ml) or urine (3 × 0·1 ml) was determined by liquid scintillation spectrometry (Beckmann Model LS 6000IC Liquid Scintillation Counter) after addition of cocktail (5 ml, Scintiverse E, Fisher Scientific). Pre-treatment plasma was assayed and determined values were subtracted as background. Samples were counted for 30 min or until counting error was less than 2%.

2.4.2 Intact fenvalerate

2.4.2.1 Procedure. Intact fenvalerate was determined in plasma from ewes treated with non-radioactive fenvalerate. The procedure employed gas chromatography/mass spectrometry (GC/MS) after conversion of fenvalerate and its internal standard (flucythrinate) to esters of 2,2-dimethyl-2-hydroxy-1-(3-phenoxyphenyl) ethanone by condensation with acetone.[7] All analyses were performed in duplicate, according to the following procedure. Twenty microlitres of internal standard (flucythrinate, 1 μg ml^{-1} in hexane) were transferred to glass tubes and evaporated. Plasma (1 ml) collected from sheep treated with fenvalerate, was then added to each tube and was followed immediately by acetone (5 ml). Samples were vortex mixed and allowed to stand at room temperature for 1 h. Acetone-precipitated protein was removed by low-speed centrifugation and sodium carbonate (1 ml, 100 g $litre^{-1}$ in water) was added to the supernatants. Samples were vortexed and allowed to stand at room temperature overnight. Acetone was then evaporated under nitrogen and the remaining aqueous portions were extracted with hexane (5 ml). Hexane layers were evaporated to dryness under nitrogen and residues were reconstituted in toluene. Two-microlitre portions were injected into the gas chromatograph.

GC/MS was performed using a Hewlett Packard Model 5989A mass spectrometer and a Model 5890, Series 2 gas chromatograph. Analyses were conducted in the negative chemical ionization mode, employing methane as ionization gas. Anions formed through cleavage of ester bonds were monitored by scanning at nominal masses corresponding to $C_{12}H_{13}O_3F_2$ (*m/z* 243, flucythrinate) and $C_{11}H_{12}O_2Cl$ (*m/z* 211 and 213, fenvalerate). Chromatographic separations were achieved on a fused silica column (SPB-1, 19 m, 0·25 mm ID, 0·25 μm film thickness). Helium carrier gas was supplied at a head pressure of 15 psi. Oven temperature was ramped from 100 to 225°C at 30° min^{-1} and then to 290°C at 10° min^{-1}.

2.4.2.2 Standard curves. Standard curve plasma samples (1 ml) containing fenvalerate (1·0, 5·0, 10·0, 50·0, 100·0 ng ml^{-1}) were assayed each day in parallel to unknown plasma. Assay accuracy was monitored by daily analysis of plasma samples containing either 5 or 10 ng ml^{-1} of fenvalerate. Samples of plasma found to contain in excess of 100 ng ml^{-1} fenvalerate were subjected to reanalysis using either 0·1 ml portions or after dilution with fenvalerate-free plasma.

2.4.2.3 Assay validation. On four different days, duplicate series of standard curve samples were prepared to contain in 1 ml of plasma 1·0, 5·0, 10·0, 25·0, 50·0 or 100·0 ng of fenvalerate. Flucythrinate (20 ng) was added, as internal standard, and samples were extracted and analysed as described above.

2.5 Pharmacokinetic modelling

Total radioactivity measured in plasma after treatment of ewes with radiolabelled fenvalerate was converted to ng equivalents of fenvalerate, on the basis of the specific activities calculated for individual dose solutions. Fenvalerate equivalents in plasma corresponding to time zero after intravenous injection were estimated by linear regression analysis of the log transforms of concentrations in plasma 15 and 30 min after treatment. Elimination-phase rate constants (β), after intravenous injection of radioactive fenvalerate or after intravenous infusion of nonradioactive fenvalerate, were determined by linear regression analysis of the log transforms of the final four plasma concentrations. Areas under the zero and first moment plasma fenvalerate/time curves were calculated by application of the linear trapezoidal rule (PCNONLIN, Version 4.1. SCI Software, Lexington, KY) and were corrected for areas from the final measurement to infinity by the standard formulae.[3] Mean residence time (MRT, corrected for infusion times), systemic clearance (Cl_S) and volumes at steady-state (V_{SS}) were calculated according to Gibaldi and Perrier.[3]

Renal clearance (Cl_R) was calculated by dividing the total radioactivity eliminated in the urine by the corresponding area under the plasma concentration–time curve.

Data are reported as arithmetic means and standard deviations except for elimination $T_{\beta}^{1/2}$ where harmonic means and pseudo-standard deviations,[8] as calculated by the jack-knife technique,[9] are reported.

3 RESULTS

3.1 Assay validation

Standard curve validation data are listed in Table 1. These data indicate that the technique was suitable for measurement of fenvalerate in plasma at concentrations between 1 and 100 ng ml^{-1}. Accuracy at the low end of this concentration range is illustrated by analysis of plasma containing known amounts of fenvalerate (5·0 and 10·0 ng ml^{-1}) (Table 1).

3.2 Reactions of ewes to intravenous fenvalerate

Each ewe treated by intravenous injection with radioactive fenvalerate exibited brief reactive episodes (5 to 10 min) characterized by salivation, tremor and hindlimb weakness. To avoid these manifestations of toxicity, ewes were subsequently exposed by intravenous infusion. Initial determinations of radioactive fenvalerate indicated rapid loss from plasma after intravenous injection. In order to compensate for lower initial levels expected after intravenous infusion the dose of fenvalerate was increased to 2 mg kg^{-1}. Ewes treated with fenvalerate by intravenous infusion did not exhibit signs of toxicity.

3.3 Total radioactivity and intact fenvalerate in plasma

Semi-logarithmic plots of fenvalerate equivalents in plasma after treatment of ewes by intravenous injection

TABLE 1
Performance Characteristics and Accuracy of the Gas Chromatographic/Mass Spectrometric Assay of Fenvalerate in Sheep Plasma (n = 4, means ± SD).

Amount (*ng ml^{-1}*)	*Response to fenvalerate* / *Response to flucythrinate (IS)*	*SD*	*CV* (%)
1·0	0·046	0·006	13·0
5·0	0·180	0·013	7·2
10·0	0·353	0·007	2·0
50·0	1·63	0·08	5·1
100·0	3·05	0·33	10·9
Amount added (ng ml^{-1})	Amount determined		
5·0	4·9 (±0·9) (n = 9)		
10·0	10·7 (±1·2) (n = 9)		

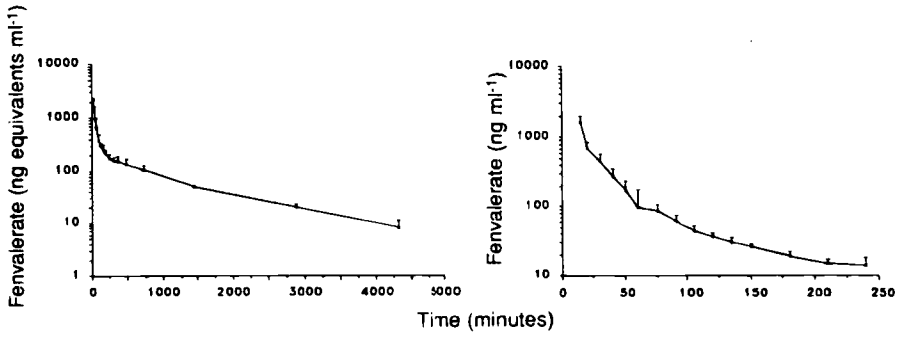

Fig. 1. Semilogarithmic plots of total radioactivity (left panel) and fenvalerate (right panel) in plasma of sheep treated with [C^{14}]fenvalerate (1 mg kg^{-1}) or non-labelled fenvalerate (2 mg kg^{-1}). Data points and error bars represent mean amounts in plasma and SD ($n = 3$).

with radiolabelled fenvalerate (1 mg kg^{-1}), or by intravenous infusion with non-radiolabelled fenvalerate (2 mg kg^{-1}), are shown in Fig. 1 (left and right panels, respectively). By 240 min after treatment, intact fenvalerate in plasma had declined to approximately 10 ng ml^{-1}, whereas the decrease in total radioactivity was slower. Pharmacokinetic parameters calculated, by noncompartmental techniques, from these data are listed in Table 2. Fenvalerate and total radioactivity associated with radiolabelled fenvalerate distributed beyond the plasma volume into peripheral compartments of similar volumes (V_{SS}). Comparisons of values calculated for MRT, β and Cl_S also indicated more rapid loss of intact fenvalerate than of total radioactivity from plasma.

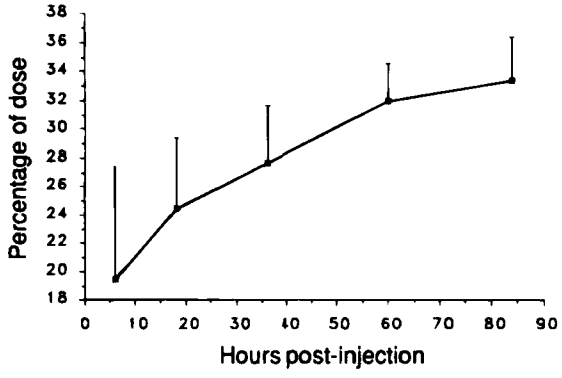

Fig. 2. Percentage of total dose excreted into urine by sheep treated by intravenous injection with [C^{14}]fenvalerate (1 mg kg^{-1}, means (±SD) $n = 3$). Data points and error bars represent mean cumulative percentages of dose eliminated into urine during each collection period. Collection times recorded on the x-axis represent mid-points of collection periods.

3.3 Radioactivity in urine

The percentage of a radioactive dose eliminated into urine is depicted in Fig. 2. The data indicate that, by 96 h after treatment, 33·3 (±3·3)% of the dose was eliminated into urine. Renal clearance of the radiolabel was 0·9 (±0·1) ml min^{-1} kg^{-1}.

4 DISCUSSION

Separation of derivatized fenvalerate and flucythrinate was achieved under the described gas chromatographic

TABLE 2

Pharmacokinetic Parameters of Intact Fenvalerate and Total Radioactivity in Plasma of Sheep Treated with either Fenvalerate (2 mg kg^{-1}) or [^{14}C]Fenvalerate (1 mg kg^{-1}) ($n = 3$, means (±SD))

Parameter	*Total radioactivity*[a]	*Fenvalerate*[b]
β (min^{-1})	0·0007 (±0·000 08)	0·0085 (±0·0018)
$T_{\frac{1}{2}\beta}$ (min)	990 (±105)[c]	82 (±14)[c]
AUC (ng min^{-1} ml^{-1})	341730 (±20000)	39430 (±4300)
MRT (min)	910 (±75)	39 (±3·0)
Cl_S (ml min^{-1} kg^{-1})	2·8 (±0·3)	51·3 (±5·9)
V_{SS} (ml kg^{-1})	2565 (±240)	2000 (±500)
Cl_R (ml min^{-1} kg^{-1})	0·9 (±0·1)	—

[a] Radioactive fenvalerate (1 mg kg^{-1}) was administered during three minutes as a single intravenous injection.

[b] Non-radioactive fenvalerate (2 mg kg^{-1}) was administered during 15 min as an intravenous infusion.

[c] Harmonic mean (±pseudo SD).

conditions. An advantage to the derivatization is that condensation with acetone removes one centre of asymmetry and the resulting chromatograms are simplified by a factor of two. Coalescence of detector response into fewer peaks also tends to increase assay sensitivity.

Measurement of total radioactivity cannot distinguish between radioactivity due to intact test substance or metabolites. However, since chemical analyses demonstrated that intact fenvalerate was rapidly lost from plasma, it is reasonable to assume that total radioactivity in plasma during its elimination phase will be more closely related to metabolites than to intact fenvalerate. The data are therefore consistent with previous reports of rapid metabolism of this pyrethroid[1,2] and also indicate that radioactivity derived from radiolabelled fenvalerate was more persistent in the plasma than was fenvalerate itself.

Systemic clearance (Cl_S) of intact fenvalerate exceeded the normal range of hepatic blood flow in sheep,[10] indicating distribution into, and clearance by, tissues other than the liver. Similarly, volumes of distribution at steady-state (V_{SS}) of intact fenvalerate and of total radioactivity were each near 2500 ml kg^{-1}, suggesting that both were distributed into similar peripheral compartments. Experiments conducted in livestock species[11,12] establish that chemical substances such as ibuprofen (2-(4-*tert*-butylphenyl)propionic acid), which closely resemble the primary metabolite (2-(4-chlorophenyl)isovaleric acid) of fenvalerate, do not distribute appreciably beyond the plasma volume and that the average MRT of ibuprofen is nearer to 90 min than to the 910 min measured for fenvalerate total radioactivity. The unexpectedly large volume of distribution determined for total radioactivity therefore suggests that a portion of metabolite formation occurred outside the central compartment, within a peripheral distribution volume occupied initially by intact fenvalerate.

This interpretation is consistent with previous reports of persistent residues of intact fenvalerate and cypermethrin in tissues of treated animals.[5,6,13–15] For example, in the rat, a portion of an oral dose of fenvalerate escaped rapid metabolism and partitioned into fatty tissue where it persisted for several weeks.[15] The depletion half-life of this residue from fat was 7–10 days, suggesting that hydrolysis of fenvalerate stored in the peripheral compartment proceeded more slowly than did elimination of metabolites returned to the central compartment. Under these special circumstances a variant of the flip-flop phenomenon can apply and the terminal-phase rate constant calculated in our experiments for elimination of total radioactivity from plasma might reflect the rate of metabolism of the fenvalerate retained in the peripheral compartment, rather than the actual rate of metabolite elimination.

Systemic clearance of total radioactivity was near 3·0 ml min^{-1} kg^{-1} and was similar to the reported Cl_S values of ibuprofen in goats and dairy cattle.[11,12] However, radioactivity recovered in urine accounted for only 30–35% of the radioactive dose, suggesting that renal clearance (Cl_R) was less than 1 ml min^{-1} kg^{-1} and in a range consistent with clearance by glomerular filtration.[16] Alternative routes of elimination were not investigated.

5 CONCLUSIONS

The data indicate slower elimination of total radioactivity than of intact fenvalerate from plasma after vascular treatment of sheep with radioactive or non-radioactive fenvalerate. This difference was apparently due to a high distributive clearance of intact fenvalerate and not to differences in volumes of distribution. In the rat, unmetabolized pyrethroids have been demonstrated to deposit into, and to be slowly released from, fatty tissues.[15] It is therefore possible that the observed rate of elimination of total radioactivity from plasma may actually represent release and degradation of fenvalerate previously deposited into fatty tissues outside the central compartment. In addition, since the elimination half-life of intact fenvalerate from plasma, after intravenous administration, was only approximately 90 min, observations of longer elimination half-lives after oral or dermal treatments may indicate 'flip-flop' absorption kinetics.

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